

PRIMITIVE NERVOUS SYSTEMS. A SENSORY NERVE-NET IN THE POLYCLAD FLATWORM *NOTOPLANA ACTICOLA*

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The nervous system of polyclad flatworms is comprised of a number of nerve tracts which radiate outwards from an anterior ganglionic mass often called the brain. These nerve tracts branch and anastomose repeatedly to form a network of strands. Two such networks have been recognized, (1) a ventral network of coarse nerves with a meshwork of finer fibers between the large strands and (2) a dorsal network of fine fibers (Hadenfeldt, 1928). Similar arrangements are found in molluscs and other invertebrates (Bullock and Horridge, 1965) but are generally less extensive. The network resembles the nerve-nets of coelenterates and echinoderms where there is diffuse conduction and information can be passed around cuts and obstructions in the nervous system. This kind of conduction has not been demonstrated in flatworms (Gruber and Ewer, 1962). On the contrary, in fact only discrete non-random conducting pathways have been demonstrated in this group. This is quite puzzling because the anatomical arrangement suggests a diffusely conducting system. Bullock and Horridge (1965) differentiate between a nerve-net and nerve plexus by considering the former as possessing diffuse conducting properties and the latter as an anatomical arrangement. The previously described discrete pathways in polyclad flatworms was surprising and the functional significance of the plexiform arrangement in this group is not clear (Horridge, 1968).

The physiological organization of polyclad nervous systems is of considerable importance from an evolutionary point of view. Polyclads are one order of platyhelminthes with clear affinities to the other major protostomous coelomates (*i.e.*, molluscs, annelids and arthropods) and are among the most primitive of these protostomes. Anatomically the nervous system is intermediate between that of coelenterates and the other protostomes, but the relationships between these groups is still controversial (Hadzi, 1963). If the flatworm nerve plexus possessed properties similar to those of the coelenterate systems then their intermediate position would be further substantiated.

The flatworm brain has considerable complexity (Best and Noel, 1969; Morita and Best, 1966; Turner, 1946) and early workers (Moore, 1923; Olmsted, 1922) demonstrated its importance for coordination of locomotory activity. Nothing is known, however, of the initiation of locomotory activity. This paper is concerned with the initiation of locomotion in the polyclad, *Notoplana acticola*, and the way that the nerve plexus transmits information to the brain. The observations made suggest that these creatures possess a sensory nerve-net.

METHODS

Animals

Mature specimens of *Notoplana acticola*, collected under rocks at Corona del Mar in Southern California were maintained in shallow plastic dishes of sea water

at room temperature, approximately 20° C. Water was changed every other day. Animals were fed adult frozen brine shrimp and were maintained in good condition for over a month.

Recordings

One of the major difficulties encountered in utilizing polyclads is their fragility. It is very difficult to attach recording devices as preparations tend to disintegrate where pressure is applied. They cannot be pinned down for dissection as the body wall tears free from the pins. The only narcotizing agent found successful was 0.36 M $MgCl_2$, but the animals tend to disintegrate when returned to fresh sea water. Consequently most data was obtained by direct observation. In only one case was a force transducer (Statham Gold Cell) successfully attached to the animal for longer than a few minutes. In this instance the transducer was held in place by attaching it to a suction electrode on the body wall. The transducer was used to measure tension of the longitudinal body musculature. Permanent records of locomotory activity were made photographically.

Stimulation

Negative going square pulses were delivered from a Grass S5 stimulator through tygon-tubing suction-electrodes applied to the dorsal surface of an animal. The electrodes remained in place for only a few minutes before the tissue under them disintegrated. Mechanical stimuli were delivered by pricking the worm with a fine (#000) insect pin.

Observations were made in 100 mm petri dishes which had a thin layer of two per cent agar on the bottom and filled with sea water. The agar acted as a cushion against occasional dragging of the animal on the substrate when it was pricked. Animals were placed in the dish for several hours before use.

Cuts were made through the body of the animal with a sharp scalpel on the day prior to use. On the observation day the cuts had started to heal but the opposing cut edges were not yet rejoined. The relationship of cuts to nerve cords was verified by staining the nervous system with the indoxyl acetate method for general esterases developed by Halton and Jennings (1964). All experiments were repeated at least ten times unless otherwise stated.

RESULTS

Ditaxic locomotion

Normal escape movements of *N. acticola* consist of alternate waves of extension and contraction. The locomotory wave begins at the anterior of the animal and passes posteriorly along the length of the body, with left and right sides of the body being out of phase with each other. The extent of movement in the front portion of the body is more vigorous than that at the rear. This kind of movement is called ditaxic locomotion. In another polyclad, *Planocera*, the brain is necessary for ditaxic locomotion (Gruber and Ewer, 1962) and if it is excised only that part of the animal directly stimulated will contract. Therefore, the initiation of ditaxic locomotion can be used to indicate that sensory information must have reached the brain.

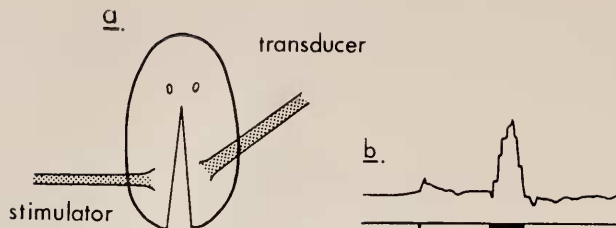


FIGURE 1. (a) A partially bisected flatworm with suction electrodes. (b) Response to a single stimulus and a train of stimuli. Intensity of each stimulus was 10 V and duration was 10 msec.

Responses to mechanical stimuli

The initial response to a pin prick at the posterior end of an animal is a small local contraction in the vicinity of the pin. If the worm is moving slowly the initial response is followed by increased anterior extension and locomotory rates. A stationary animal does not usually start to move unless the stimulus is repeated. A second jab within a few seconds of the first causes the animal to extend its anterior margin on one side and move away. Sometimes 3 or 4 pricks may be necessary to accomplish this.

Responses to electrical stimuli

Electrical stimuli do not elicit ditaxic locomotion. If they are applied between a single electrode on the posterior part of the animal and a ground in the surrounding water only localized twitches are produced, no matter how many stimuli are given. Similarly, shocks applied between two widely separated electrodes may produce considerable contraction between the electrodes without causing the animal to move. Electrical stimulation does cause a certain amount of propagated activity. In *Planocera* we showed that stimuli could be conducted from one side of the body to the other provided the brain was intact (Koopowitz and Ewer, 1970). When a specimen of *Notoplana* was split up the midline from the posterior margin to just behind the brain, electrical stimulation of one side also caused contractions to occur on both sides of the animal. In one preparation it was possible to measure the tension on the one side while stimulating the other side electrically (Fig. 1). Although mechanical stimulation can evoke ditaxis, electrical stimulation does not. Neither single nor multiple stimuli produce a response other than longitudinal contractions. This data indicates that activity is promulgated from the one side of the animal to the other, probably through the brain.

Decerebrate animals

The brain is necessary to initiate locomotion. Animals from which the brain had been removed did not respond to mechanical stimulation. Instead they produced local twitches, reminiscent of those elicited by electrical stimulation.

Lesions posterior to the brain

Stimulating an animal behind a cut, which is posterior to the brain and has severed both of the major longitudinal nerve cords, still initiates ditaxic locomotion

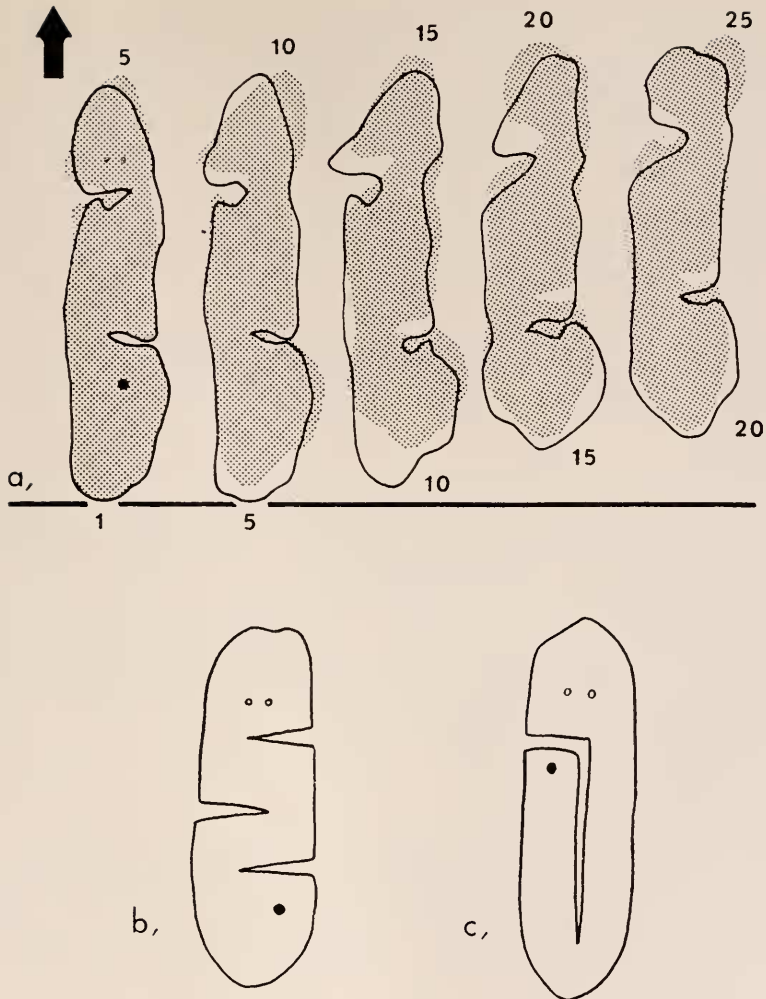


FIGURE 2. (a) Tracings of the first movements in ditaxic locomotion. This particular animal had two cuts which severed the longitudinal cords. Traced from a series of ciné film frames. Image with the solid contour represents the frame numbered at the bottom while the dotted area is the animal's position five frames later. Film speed was 16 frames per second. (b) Diagram shows the position of three interdigitating cuts through the animal's body; (c) position of cuts made to demonstrate posterior propagation of the stimulus. Solid dot is site of stimulation.

and the portion anterior to the cut is used for ditaxis. The part behind the cut does not appear to be involved with motor activity, on that side of the body.

It is also possible to initiate locomotion with mechanical stimuli delivered behind the most posterior of two interdigitating transverse cuts. Figure 2a illustrates the response evolved from a preparation in which the incisions were from

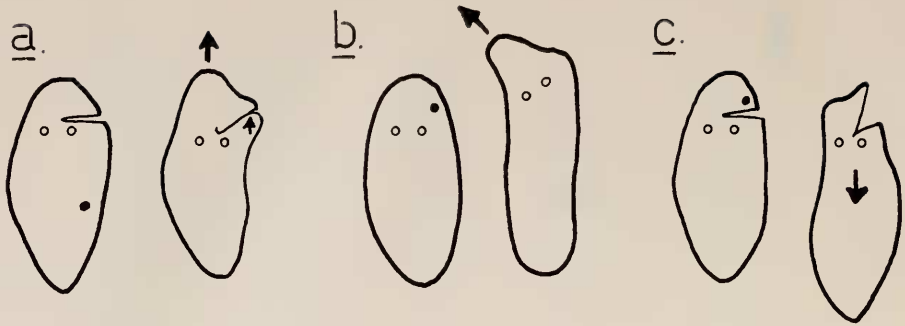


FIGURE 3. (a) The initial movement following posterior stimulation with an anterior cut. (b) Normal avoidance response to an anterior stimulus. (c) Avoidance response to an anterior stimulus after an anterior cut. Drawings were made from life.

opposite sides of the animal and across the midline so that both longitudinal nerve cords were severed. The contraction pattern is typical of ditaxic locomotion. Ditaxic locomotion can be elicited when three interdigitating cuts are made (Fig. 2b) but movement could not be evoked in all preparations. Conduction did not occur around more than three overlapping cuts.

An interesting situation occurs when a cut is made behind the brain and continued posteriorly along the midline for most of the animal's length (Fig. 2c). Stimulation of the anterior portion of this strip results in ditaxic locomotion at the anterior end of the animal. Therefore the information is conducted posteriorly before being conducted anteriorly to the brain.

Lesions anterior to the brain

When a cut is made from the antero-lateral margin to a point midway in front of the brain (Fig. 3a), animals prodded behind the brain move ditaxically. However, the flap of anterior margin produced by the cut, does not take part in the process. The uncut portion performs normally but on the lesioned side muscular extension only occurs behind the cut. Movement waves are propagated posteriorly from this point.

If an intact animal is prodded along the anterior margin it twists to the opposite side before moving away (Fig. 3b). A cut made between the brain and the anterior stimulus site results in a different kind of reaction (Fig. 3c). The animal no longer performs the twisting avoidance reaction but retracts and backs away.

DISCUSSION

The major finding of this study is the presence of an apparent sensory nerve-net in *Notoplana*, a turbellarian. This system resembles those classically designated as nerve-nets (Bullock, 1965) with isopolar diffuse conducting systems. A physiological organization of this type has not been demonstrated in this group of animals before and is of some importance with regard to current concepts about the evolution of nervous systems. It should be mentioned, however, that two other types of conducting systems could be invoked to explain the results obtained

here and neither of these can be completely excluded as the responsible systems. Conduction around lesions could occur in either the muscle layers or the epithelium. Possible anatomical grounds for muscle-muscle conduction have been found in tight junctions between adjacent sarcoplasmic membranes (Chien and Koopowitz, 1972), which could act as electrical synapses. However, for a number of reasons, muscular propagation is probably not responsible. First, one might expect a wave of contraction or extrusion to accompany conduction. This is not the case. Secondly, localized or extensive contractions caused by either mechanical or electrical stimuli do not themselves lead to ditaxic locomotion. Thirdly, it is difficult to envisage how information propagated in the muscle layers could be transferred to the brain—even if it were to reach the region of that organ. Possible pressure in stretch receptors in the muscles could translate contraction into removal activity, but one would expect these to be scattered throughout the organism and activated close to the site of stimulation.

Neuroidal conduction might feasibly be involved in the observed results. Epithelial, or neuroid, conduction is well known in animals as diverse as coelenterates (Mackie, 1970) and larval amphibia (Roberts, 1969). However, the problem of transferring the information to the brain from the epithelium remains. Jha and Mackie (1967) have shown at the ultrastructural cell level how the ectoderm might connect to the nervous system in *Cordylophora*, a hydrozoan. But, as yet, epithelial-neuronal connections are not known in the Turbellaria. Perhaps the best evidence that epithelial conduction is not involved comes from the different behavioral responses obtained from comparison between stimulation anterior to or behind the brain. One would not expect anterior stimulation to evoke backing away, while posterior stimulation causes forward locomotion if the same epithelial system was involved. It is difficult to see how an epithelial conducting system could differentiate between the positional information in the sites of the two stimuli.

The simplest hypothesis to explain the present results would be by invoking a diffuse conducting neural network. However, if the nerves are responsible then one might question why ditaxic locomotion cannot be evoked by electrical stimulation. Other attempts have been made to demonstrate diffuse conduction in the large nerve plexus of the polyclad *Planocera* (Gruber and Ewer, 1962; Ewer, 1965). These authors found that conduction (initiated by electrical stimulation to the brain) only occurred along direct routes to the brain and concluded that a diffusely conducting nerve-net did not exist. Perhaps they did not have the correct stimulus for evoking activity in the diffuse conducting systems. A similar kind of finding is reported in the echinoderms, where mechanical stimulation of the radial nerve cord produces impulses but electrical stimuli are ineffective (Cobb and Laverack, 1966). Pentreath and Cobb (1972) suggested that electrical stimuli might not elicit a response in echinoderms if the axons are small and highly insulated. This might hold for the sensory nerves in flatworms as well.

At present one cannot determine which part of the nervous system might be responsible for conduction around the lesions. Besides the two submuscular plexuses, there is also the possibility of a fine subepithelial or epithelial nerve-net. There are a number of reports in the literature of such nerve-nets in the turbellarians. Lentz (1968) has described an epithelial net in fresh-water planarians, but these networks have not been convincingly demonstrated in polyclads, either at

the light or electron microscope level. Even in the simpler orders of the class an epithelial net appears uncommon (Bullock and Horridge, 1965), if indeed it actually occurs. One wonders if perhaps overlapping terminal branches of sensory cells might have been misinterpreted as an epidermal network by some of the earlier workers.

The functional significance of the anatomical network is puzzling. If a nerve-net has indeed been demonstrated in this work, then it appears to be confined to only the sensory system. Cutting nerves leading to anterior motor regions leave that part flaccid and incapable of joining in locomotory behavior patterns. It is also clear, however, that the entire sensory system is not arranged as a physiological nerve-net; not even for single modalities such as mechanoreception. Avoidance responses from the anterior edge of the animal indicate that very specific pathways are involved. Recruitment of certain set pathways is obviously important when it is necessary for an animal to localize the site of a stimulus if it must avoid the stimulus source. This is not important for stimuli behind the brain as normal locomotion will take the animal away from the source of irritation. The presence of a back-up system such as the nonspecific anterior system which causes the animal to back away from the stimulus has obvious selective advantages. But even if these nonspecific systems are based on an anatomical nerve-net it is difficult to see why this should dictate the form of the comparatively massive plexiform nervous system which exists. It is probably more reasonable to assume that some other, as yet unknown, function is responsible for the selective advantage that maintains this anastomosing arrangement.

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SUMMARY

(1). The response to mechanical stimuli in the polyclad flatworm, *Notoplana acticola*, is the initiation of ditaxic locomotion. The response to electrical stimuli is local contraction.

(2). Animals will respond to mechanical stimuli with ditaxic movements even if a series of cuts are made so that the stimulus must be propagated around lesions as in a nerve-net.

(3). Only the sensory side of the system is organized as a diffusely conducted system; motor control involves direct connections to the brain.

(4). Sensory stimuli that convey information about the location of a stimulus on the body also require direct routes.

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